REMARKS/ARGUMENTS

The Pending Claims

Claims 8-13 and 35 are pending and are directed to a method of producing a rat embryonic stem (ES) cell.

Amendments to the Claims

The claims have been amended to further clarify the claimed subject matter.

Claim 8 has been amended to recite that the rat blastocyst is cultured in a leukemia factor (LIF)-free culture medium, which is supported by the specification at, for example, page 17, lines 29-32, and page 41, lines 21-33.

Claims 12 and 13 have been amended to recite the full-length term for rLIF.

Claim 35 is new and recites a feature that previously was recited in claim 8.

Claims 1-7, 14-30, and 32-34 have been canceled as directed to non-elected subject matter in response to the restriction requirement. Applicants reserve the right to pursue the subject matter of the canceled claims in one or more divisional, continuation, continuation-in-part, or other applications.

No new matter has been added by way of these amendments to the claims.

Summary of the Office Action

The Office makes final the restriction requirement and withdraws non-elected claims 1-7, 14-30, and 32-34 from consideration.

The Office objects to claims 12 and 13.

The Office rejects claims 8-13 under 35 U.S.C. § 112, second paragraph, as allegedly indefinite.

The Office rejects claims 8 and 10-12 under 35 U.S.C. § 102(b) as allegedly anticipated by Vassilieva et al. (*Experimental Cell Research, 258*: 361-373 (2000)). The

Office also rejects claims 8, 10, and 12 under 35 U.S.C. § 102(b) as allegedly anticipated by Loring (WO 99/27076).

The Office rejects claims 8 and 13 under 35 U.S.C. § 103(a) as allegedly obvious in view of either Vassilieva et al. or Loring in view of Takahama et al. (*Oncogene, 16*: 3189-3196 (1998)). The Office also rejects claims 8 and 9 under 35 U.S.C. § 103(a) as allegedly obvious in view of either Vassilieva et al. or Loring in view of Price et al. (WO 98/30679).

Reconsideration of these objections and rejections is hereby requested.

Discussion of the Claim Objections

The Office objects to claims 12 and 13 because the abbreviation of "rLIF" is not accompanied by the full-length name. Claims 12 and 13 have been amended to recite "rat leukemia inhibitory factor (rLIF)." Applicants believe that the claim objections are moot in view of the amendments to the claims.

Discussion of the Indefiniteness Rejections

The Office contends that several phrases in claim 8 lack antecedent basis. Additionally, the Office requires clarification of the culture conditions of rat blastocysts (in step (A)) and the dissociated inner cell mass (in step (B)).

Claim 8 has been amended to further clarify the claimed subject matter. Applicants believe that the rejections are moot in view of the amendments to claim 8.

Discussion of the Anticipation Rejections

The Office contends that each of Vassilieva et al. and Loring discloses a method for obtaining rat ES cells that encompasses the subject matter of one or more of claims 8 and 10-12. These rejections are traversed for the following reasons.

Claim 8, as amended, recites a method of producing a rat ES cell, which comprises (A) culturing a rat blastocyst in a LIF-free culture medium to form an inner cell mass in the blastocyst, (B) dissociating the inner cell mass, wherein the dissociated inner cell mass is in a cell aggregate state, (C) culturing primary ES cells resulting from a culture of the dissociated

inner cell mass until the primary ES cells can be passaged, (D) dissociating the primary ES cells, which can be passaged, wherein the dissociated primary ES cells are in a cell aggregate state, and (E) culturing the dissociated primary ES cells to establish an ES cell. Thus, claim 8 (and, therefore, claims 10-12 dependent thereon) requires that a rat blastocyst is cultured in a *LIF-free* culture medium to form an inner cell mass.

A high concentration of LIF is used for the formation of rat inner cell masses in both Vassilieva et al. (20 ng/mL of human LIF) and Loring (100-200 units/mL of LIF). Therefore, the cited references do not anticipate the subject matter of claims 8 and 10-12.

For these reasons, Applicants request that the anticipation rejections be withdrawn.

Discussion of the Obviousness Rejections

The Office contends that it would have been obvious to one of ordinary skill in the art to arrive at the invention in view of the disclosures of (a) either Vassilieva et al. or Loring in combination with (b) either Takahama et al. or Price et al. The obviousness rejections are traversed for the following reasons.

For subject matter defined by a claim to be considered obvious, the Office must demonstrate that the differences between the claimed subject matter and the prior art "are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains." 35 U.S.C. § 103(a); see also *Graham v. John Deere Co.*, 383 U.S. 1, 148 U.S.P.Q. 459 (1966). The ultimate determination of whether an invention is or is not obvious is based on certain factual inquiries including: (1) the scope and content of the prior art, (2) the level of ordinary skill in the prior art, (3) the differences between the claimed invention and the prior art, and (4) objective evidence of nonobviousness. *Graham*, 383 U.S. at 17-18, 148 U.S.P.Q. at 467.

Consideration of the aforementioned *Graham* factors here indicates that the present invention, as defined by the pending claims, is unobvious in view of the cited references.

As regards the scope and content of the prior art, the Office contends that Vassilieva et al. and Loring disclose a method for obtaining rat ES cells. The Office further contends

that Price et al. discloses the use of serum replacement medium to support the growth of ES cells in order to avoid problems associated with the use of serum, and that Takahama et al. discloses the cloning of cDNA encoding rat LIF and demonstrated that culturing with rat LIF maintained the undifferentiated state of rat ES cells.

For purposes of the analysis here, and for the sake of argument, the level of ordinary skill can be considered to be relatively high, such that a person of ordinary skill in the art would have an advanced degree and/or several years of experience in the relevant field.

The present invention, as defined by the pending claims, is directed to a method of producing a rat ES cell, which comprises (A) culturing a rat blastocyst in a LIF-free culture medium to form an inner cell mass in the blastocyst, (B) dissociating the inner cell mass, wherein the dissociated inner cell mass is in a cell aggregate state, (C) culturing primary ES cells resulting from a culture of the dissociated inner cell mass until the primary ES cells can be passaged, (D) dissociating the primary ES cells, which can be passaged, wherein the dissociated primary ES cells are in a cell aggregate state, and (E) culturing the dissociated primary ES cells to establish an ES cell.

As discussed above with respect to the anticipation rejections, Vassilieva et al. and Loring do not disclose culturing a rat blastocyst in a *LIF-free* culture medium to form an inner cell mass, as required by the pending claims. This deficiency is not remedied by Takahama et al. or Price et al.

Indeed, prior to the claimed invention, it was a commonly held belief that LIF must be added to a medium to form an inner cell mass from a blastocyst. The inventors surprisingly discovered that that rat inner cell masses can be efficiently formed by culturing rat blastocysts in a LIF-free medium (see, e.g., page 41, lines 21-33, of the specification).

As described in the Declaration Under 37 C.F.R. § 1.132 of Takahiro Ochiya (submitted herewith), the use of a LIF-free medium results in the formation of more inner cell masses than when a LIF-containing medium is used. In particular, inner cell masses were formed in 3 out of 10 rat blastocysts (30%) using a LIF-free medium, while only 1, at most, inner cell mass was formed from 6 blastocysts (<17%) when rLIF was added to the culture medium in a range of 100-5000 units (see page 2 of the Rule 132 Declaration).

Applicants note that prior to the claimed invention the establishment of rat ES cells had not been reported even though various other mammalian ES cells had been successfully established following the first report in 1981 of mouse ES cell establishment (see, e.g., page 3, lines 20-24, of the specification). The inventors determined suitable culture and passage conditions and succeeded in establishing rat ES cells capable of forming a chimeric rat. Since the development of rat ES cells is essential for the production of a knockout rat, the claimed invention provides the first means for producing various experimental rat models.

Applicants further note that rat ES cells produced by the inventive method demonstrate superior effects when compared to the cells of Loring and Vassilieva et al. As shown in Example 4 of Loring et al., BNRB-1 cells derived from rat embryo cannot form embryoid bodies before the co-culture with mouse ES cells. Thus, the method disclosed in Loring et al. requires an additional step for co-culturing multipotent cells with non-rat (e.g., mouse) ES cells in order to confer embryoid body-forming ability (pluripotency) on the multipotent cells. In contrast, rat ES cells obtained by the inventive method are capable of forming embryoid bodies, even though the method does not comprise the step for co-culturing with non-rat ES cells (see, for instance, Example 2 of the specification). Furthermore, the rat pluripotent cells obtained by the co-culture with mouse ES cells are not recognized by the art as true rat ES cells, since their chimeric rat-producing ability has not been confirmed.

As regards Vassilieva et al., the cells obtained by the method disclosed therein merely were confirmed to express some marker genes and antigens for ES cells. It is unknown if the cells are pluripotent and capable of producing a chimeric rat. Indeed, the authors themselves call the cells "ES-like cells" rather than "ES cells."

Considering all of the *Graham* factors together, it is clear that the present invention – as defined by the pending claims – would not have been obvious to one of ordinary skill in the art at the relevant time in view of the combined disclosures of the cited references. Accordingly, the obviousness rejections should be withdrawn.

Conclusion

Applicants respectfully submit that the patent application is in condition for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

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